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14. ABSTRACT

Prostate cancer progresses from androgen-dependent to androgen-independent state. The androgen receptor (AR) isexpressed throughout progression. We would like to understand the AR role in this progression. Using lox-Cre methodology,we have generated mice in which AR function is abolished in the entire animal (ARKO) or tissue specific manner. We willgenerate mice with ARKO in prostate only or in different stages to be used to study prostate cancer progresses. Our Aimsfollow. 1: Generate mice lacking functional AR in prostate epithelium. 2: Generate inducible ARKO mouse line to be used todetermine potential effect of androgen in absence of AR on prostate growth/maintenance. 3: Determine AR role in prostatecancer development/progression by crossing ARKO mice with TRAMP mice to examine AR role in TRAMP induced prostatecancer and permit determination of points in prostate cancer requiring AR function. 4: Determine AR role in tumorigenicity ofandrogen-dependent and androgen-independent ARKO prostate cancer cell lines. The effect of AR loss in these cells will beexamined for ability to generate/promote tumors in mice. This year we generated mice with ARKO in the prostate epitheliumand will be able to continue the other aims in the proposal in the coming years.

15. SUBJECT TERMS
ANDROGEN RECEPTOR, KNOCKOUT GENES, PROSTATE CANCER

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Table of Contents

Introduction and Body:

A summary of this proposal was the following statement:

While androgen receptor (AR) activity is known to be important in the development and maintenance of the normal prostate and in prostate cancer, several aspects of AR function in the prostate have not been able to be addressed until now due to a lack of appropriate animal models. We have developed mice with a floxed AR exon 2, enabling us to inactivate AR in a tissue specific or temporally specific manner. Using these mice, this proposal seeks to address several aspects of the role of AR in the prostate that could not previously be addressed with Tfm mice or by treatment with antiandrogens. The role of epithelial AR function in communication with the stroma of the fully formed prostate will be addressed in Specific Aim 1. This will allow an initial determination of the androgen regulated stromal factors that may contribute to epithelial cell survival. The possible role of recently described rapid nongenomic effects of androgens in the prostate will be addressed using an inducible system to render AR non-functional in the prostate at specific developmental stages in Specific Aim 2. The role of AR at specific stages of prostate cancer progression will be examined in a mouse model system in Specific Aim 3. In Specific Aim 4, suppression of AR expression in prostate cancer cell lines will be examined for the effect of prostate cancer cell tumorigenicity.

The Specific Aims and Sub Aims of the proposal are the following.
Aim 1: To generate male mice specifically lacking a functional AR in the prostate epithelium.
        Aim 1a: To generate mice lacking a functional AR in the prostate.
        Aim 1b: To examine the effect of loss of AR function in the prostate.

Aim 2: To generate an inducible ARKO mouse line.
        Aim 2a: To generate floxAR mice that carry an inducible Cre transgene.
        Aim 2b: To examine the effect of the inducible loss of AR on the development and maintenance of the prostate.

Aim 3: To determine the role of AR in prostate cancer development and progression in mice by crossing the ARKO mice generated in Specific Aims 1 and 2 with TRAMP transgenic mice.
        Aim 3a: To generate mice lacking prostate specific AR function in a mouse model of prostate cancer.
        Aim 3b: To determine the effect of loss of prostatic AR function in the initiation and progression of prostate cancer.
        Aim 3c: To generate mice with an inducible loss of AR function in a mouse model of prostate cancer.
        Aim 3d: To determine the effect of loss of AR function at specific points in the initiation and progression prostate cancer.

Aim 4: To determine the role of AR in the tumorigenicity of androgen dependent and androgen independent AR knockout prostate cancer cells.
        Aim 4a: To generate prostate cancer cell lines lacking AR by somatic homologous recombination
        Aim 4b: To generate prostate cancer cell lines lacking AR by RNA interference (RNAi).
        Aim 4c: To determine the effect of the removal of AR activity on tumor formation and growth in mice.

Our proposed schedule for completion of this proposal included the following tasks during the first 12 months.
        Task 1 (1-12 month)**:** To generate mice lacking a functional AR in the prostate.
        Task 3 (1-12 month)**:** To generate floxAR mice that carry an inducible Cre transgene.

of prostate cancer.

Task 7 (1-13 month): To generate mice with an inducible loss of AR function in a mouse model of prostate cancer

Task 9 (1-18 month): To generate prostate cancer cell lines lacking AR by somatic homologous recombination.

Task 10 (1-12 month): To generate prostate cancer cell lines lacking AR by RNA interference (RNAi).

Key Research Accomplishments and Reportable Outcomes:

Our progress and completion of Tasks 1, 3, and 5 can be best seen in the following description of the Figure below written for a manuscript being prepared for publication.

Previous studies using prostate tissue recombination demonstrated that androgen receptor (AR) in stromal cells, but not in epithelium plays essential role for the prostate development. Epithelia of testicular feminization (Tfm) mice with mutant AR forms prostatic buds when they are recombined with normal mesenchyme in the presence of androgens; in contrast, normal epithelia fail to form prostatic buds when they are recombined with Tfm mesenchyme. These results suggest stromal but not epithelial AR plays essential roles for the early prostate development, and androgen-induced paracrine growth factor signals from the mesenchyme might induce and pattern the adjacent epithelial development. Yet so far, no single growth factor has been identified that can completely restore the stromal AR function, suggesting multiple growth factors, including those in the epithelium might all contribute to the prostate development.

Most of the epithelial-stromal recombination studies discontinues experiments at 4th week, and therefore prevent further study of the epithelial AR roles in the adult prostate development. Mice lacking both stromal and epithelial ARs fail to develop prostate, which also prohibits our study of AR roles in prostate development. Here we generate prostate epithelium-specific AR knockout (pes-ARKO) mice via mating ARR2PB-Cre mice with flox-AR mice. The successful generation of their offspring (pes-ARKO) represents the first available *in vivo* animal model to study the influence of loss of AR in adult prostate epithelia.

The probasin expression in the wild type (WT) prostate was first detected at the 3rd week after birth. At the 4th week, the initial expressed probasin was confined in the cytoplasm of the prostate epithelia when the glandular structure still remains immature (Fig. 1a). At the end of puberty (5-6 week) the probasin was secreted into lumen of the matured prostate gland (Fig. 1b), and this protein secretory function remained intact in the adult mice (12 week) (Fig. 1c). In contrast, the probasin expression in pes-ARKO mice was similar to WT mice till the 5th week (the time AR starts to be knocked out), but gradually reduced expression until it was almost undetectable in the lumen of the adult prostate at the 24th week (Fig. 1d-f, 7c).

AR in WT mice was located in the nuclei of secretory epithelial cells and in the scatter stromal cells (Fig. 1g). In contrast, while pes-ARKO mice had normal AR expression pattern in the stromal cells from early to late stages of prostate development, the AR expression in the secretory epithelial cells was gradually decreased from the 6th week to nearly complete loss at the 24th week (Fig. 1h-k).
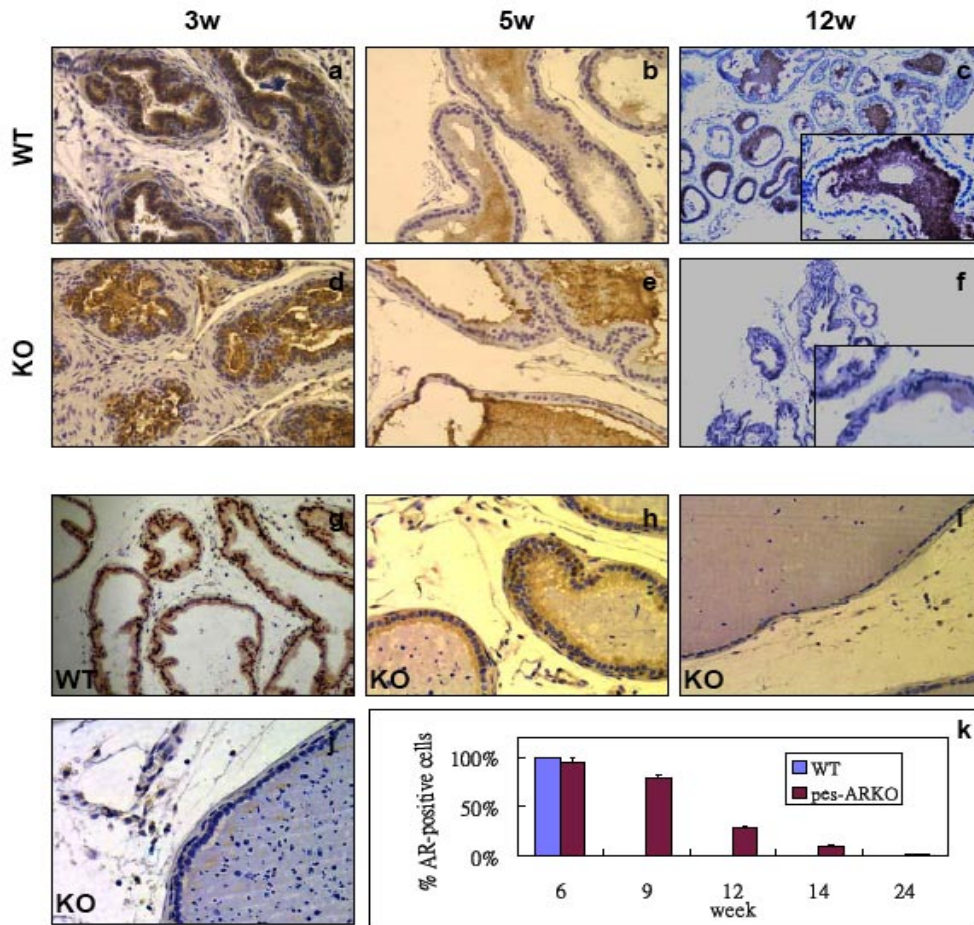
Fig. legend

Changes in expression of probasin and AR in prostate of pes-ARKO mice. IHC staining with probasin Ab (a-f) and AR Ab (g-j). Probasin expression in the cytoplasm starts at the 3rd weeks (a, d). At the 5th week, WT and pes-ARKO reach maturation and secret probasin into the lumen (b, e). At the 12th, pes-ARKO showed decreased probasin secretion (c, f). WT prostate expressed AR in both epithelial and stromal cells (g). In the pes-ARKO there was gradually loss of AR in the epithelial cells (h, 6w; i, 14w; j, 24w). The percentage of AR positive epithelial cells in the pes-ARKO mice gradually decreased (k).

Key Accomplishment List

•Task 1 (1-12 month)**:** We generated mice lacking a functional AR in the prostate epithelium (pes-ARKO).

•Task 3 (1-12 month)**:** We generated floxAR mice that carry an inducible Cre transgene (ind-ARKO).

•Task 5 (1-12 month)**:** We demonstrated that our pes-ARKO mice lack prostate specific AR function (Fig. 1).

•Task 7 (1-13 month)**:** To generate mice with an inducible loss of AR function in a mouse model of prostate cancer (ind-pes-ARKO). This task has been initiated but the proper genotypes are slowly being raised and the inducible model takes time before it can been proven and will be reported in the next annual report.

•Task 9 (1-18 month)**:** To generate prostate cancer cell lines lacking AR by somatic homologous recombination. This task was initiated, but no statistically significant results during the reporting period.

•Task 10 (1-12 month)**:** To generate prostate cancer cell lines lacking AR by RNA interference (RNAi). This task was initiated, but no statistically significant results during the reporting period.

Reportable Outcomes:

- flox AR mice
- pes-ARKO mouse model

Conclusions:
The other tasks for the first year are well on the way to completion and will be reported with study results in the next annual report. We have made very promising progress in this first year even though there is only the one figure presented in this annual report. The inducible mouse model, and other cell lines that are in production are very time consuming and difficult to produce, but are being produced in enough numbers for further experiment within the second year of the grant. All of the initiated tasks will allow us to continue with the other tasks towards completion of the entire project within the 3 years.

References and Appendices: None